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SHORT COMMUNICATION

High serum total cholesterol is a long-term cause of osteoporotic fracture

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Abstract
Summary Risk factors for osteoporotic fractures were evaluated in 1,396 men and women for a period of 20 years. Serum total cholesterol was found to be an independent osteoporotic fracture risk factor whose predictive power improves with time.
Introduction The purpose of this study was to evaluate long-term risk factors for osteoporotic fracture.
Methods A population random sample of men and women aged 25–64 years (the Gothenburg WHO MONICA project, N=1,396, 53% women) was studied prospectively. The 1985 baseline examination recorded physical activity at work and during leisure time, psychological stress, smoking habits, coffee consumption, BMI, waist/hip ratio, blood pressure, total, HDL and LDL cholesterol, triglycerides, and fibrinogen. Osteoporotic fractures over a period of 20 years were retrieved from the Gothenburg hospital registers. Poisson regression was used to analyze the predictive power for osteoporotic fracture of each risk factor.
Results A total number of 258 osteoporotic fractures occurred in 143 participants (10.2%). As expected, we found that previous fracture, smoking, coffee consumption, and lower BMI each increase the risk for osteoporotic fracture independently of age and sex. More unexpectedly, we found that the gradient of risk of serum total cholesterol to predict osteoporotic fracture significantly increases over time (p=0.0377).
Conclusions Serum total cholesterol is an independent osteoporotic fracture risk factor whose predictive power improves with time. High serum total cholesterol is a long-term cause of osteoporotic fracture.

Keywords Cholesterol · Gradient of risk
Osteoporotic fracture · Risk factor

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Introduction

Osteoporotic fractures increase mortality rates and place a progressively larger burden on healthcare resources [1]. In the case of hip fracture, mortality is directly dependent on the fracture per se [2]. In a recent study, we reported that hard cardiovascular endpoints constitute strong predictive risk factors for hip fractures among elderly men [3]. This

finding prompted us to explore links between osteoporotic fractures and cardiovascular risk factors among a younger cohort. To this end, we conducted a 20-year long prospective study in a population of men and women aged between 25 and 64 years, in which blood lipids, blood pressure, fibrinogen, and lifestyle factors were analyzed with respect to their ability to predict osteoporotic fracture.

Materials and methods

Participants

Gothenburg, with a population of ~450,000, is the second largest city in Sweden. In 1985, 1,000 men and 1,000 women of Caucasian origin, aged between 25 and 64 years, were selected at random from the population census of the city and invited to participate in the World Health Organization MONItoring of trends and determinants in CArdiovascular disease (WHO-MONICA) project. Out of the invited 2,000 subjects, 1,396 (53% women) participated at the 1985 baseline examination [4]. Participation rates did not differ between the sexes and varied from 65% among subjects aged 25–34 years to 74% among subjects aged 55–64 years.

Fractures

Records of X-ray-verified fractures deemed to be of osteoporotic origin (upper arm, wrist, ankle, leg, hip, pelvis, rib, vertebrae, and foot, International Classification of Diseases (ICD) 9 codes 805–825 and E885–E888, and ICD 10 codes S07, S12, S22, S32, S42, S52, S62, S72, S82, S92) during 22 years (1985–2007) were retrieved from the Gothenburg hospital registers via the National Board for Health and Social Welfare, Stockholm, Sweden.

Lifestyle factors

Past and present health status, smoking habit, coffee consumption, medication, psychological stress, and physical activity during work and leisure time were assessed with questionnaires at the time of the 1985 baseline examination.

Smoking habits were coded as (1) smoker, (2) ex-smoker, and (3) non-smoker. A non-smoker was a person who had never smoked, or occasionally smoked less than one cigarette per day. Ex-smokers were participants that no longer smoked, but in earlier life would have qualified as regular smokers for a period of at least 1 month. Smokers were asked not to smoke during the mornings of their examinations. Coffee consumption was estimated as average number of cups per day. Physical activity during work was graded as follows: grade (1) work denoted predominantly sedentary, grade (2) work included some walking and standing but no stairs or heavy lifting, grade (3) work involved walking including stairs, or walking uphill and/or lifting heavy objects, and grade (4) work corresponded to heavy physical labor. Physical activity during leisure time was graded as follows: grade (1) denoted predominantly sedentary, i.e., reading or watching television; grade (2) included moderate activity like walking, riding a bicycle, and/or light garden work for at least 4 h per week; grade (3) involved regular exercise such as running, swimming, tennis, heavy gardening at least 2 to 3 h per week; and grade (4) was defined as regular athletic training and/or participation in competitive sports several times per week. The grading was based on questionnaires previously used to assess the relationship between physical activity and risk of myocardial infarction [5, 6]. Psychological stress, defined as feeling tense, irritated, nervous, anxious, or experiencing sleep disturbances related to problems at home or at work, was graded (1)–(6), with (1) denoting no stress experience, (2) experience of rare occasions of some stress, (3) some periods of stress experience during the last 5 years, (4) several periods of stress experience during the last 5 years, (5) continuous experience of stress during the last year, and (6) continuous experience of stress during the last 5 years.

Blood pressure

Right arm blood pressure was measured twice to the nearest 2 mmHg following a 10-min rest in sitting position. Disappearance of Korotkoff sounds (phase V) was used to determine diastolic blood pressure. A random zero blood pressure machine (Hawksley & Sons, Lancing, UK) was used. A cuff size corresponding to the circumference of the right arm was chosen. A single observer performed the measurements.

Anthropometry, hypertension, and diabetes

Body weight was measured to the nearest 0.1 kg, in the fasting state with the subject barefoot and in underwear. Body height was measured barefoot, and to the nearest 1.0 cm. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Waist circumference was measured using a tape measure midway between the lowest rib margin and the iliac crest with the subject in standing position. The hip circumference was measured over the widest part of the gluteal region and the waist/hip ratio was calculated. A single person performed all measurements. Hypertension was defined as a mean blood pressure >160/90 or treatment for hypertension [4]. Diabetes was defined as known treatment for diabetes.

Blood samples

Following overnight fast, venous blood samples from an antecubital vein were drawn sometime between 8:00 am and

10:00 am. Serum total cholesterol, as well as low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides, and fibrinogen were measured for all 1,396 participants. After centrifugation, all plasma samples were frozen and stored at −70°C until analysis, which was performed within a year. Concentrations of total and HDL cholesterol and triglycerides were determined enzymatically (Boehringer Mannheim, Mannheim, Germany). LDL cholesterol was determined as previously described by Friedewald [4]. Plasma fibrinogen was analyzed with nine volumes of blood drawn into one volume of 0.13 M trisodium citrate, according to a polymerization method.

Medications

Estrogen hormone replacement therapy (HRT) was used by 33% of women aged 45 or above at the time of the baseline examination. Calcium and vitamin D supplementation was used by <1% of the participants, corticosteroids were used by <1%, and lipid-lowering medication was used by <1% (three men). Antihypertensive agents were taken by 7% of the participants, and 3% took peroral antidiabetic medications. Except for calcium/vitamin D, none of the participants used a specific antiosteoporotic agent at the time of the baseline examination.

Ethical considerations

The study had been approved by the Ethics Committee at University of Gothenburg and the National Data Inspection Board. Each participant was informed about the aim of the study in writing, and all participants gave their written informed consent.

Additional approval to collect data for osteoporotic fractures for 20 years was given by the National Data Inspection Board.

Statistical methods

Mean value, standard deviation (SD) and confidence intervals (CI) were calculated using conventional methods. Student's *t* test and chi-square were used for a brief comparison between groups.

A special type of Poisson regression analysis was performed in order to estimate continuous hazard functions, from which it is possible to calculate the hazard ratio (HR) over time for osteoporotic fracture for each risk factor taking age and sex into account [7]. Only the first fracture is included in the analyses for subjects that sustained more than one fracture. To characterize the efficacy of the risk factor as a predictor of fracture, the gradient of risk per one SD was used [8]. The gradient of risk per one SD of a predictor is the HR between two individuals, who differ by one SD with respect to the predictor, but are equal with respect to other variables. A p value of <0.05 (two-sided test) was considered significant.

A hazard function describes the momentary risk of an event. The momentary risk for osteoporotic fracture is expected to depend on current bone quality variables, and previous values of such variables are not expected to contribute appreciably to the risk. This implies, together with some general assumptions, that the predictive power of a bone quality variable determined at a baseline examination normally decreases with time. One might say that a bone quality variable constitutes a predictor of osteoporotic fractures if it is able to predict future bone quality. A risk variable is thus more strongly related to future bone quality than to present bone quality if its predictive power increases with time. A risk variable that slowly affects bone quality could behave in such a manner.

Results

The main objective of this study was to examine to what extent factors measured at the baseline examination predict the incidence of osteoporotic fractures over time. Anthropometric data, life style factors, blood pressure, lipids, and fibrinogen in participants with and without osteoporotic fractures are listed in Table 1. In general, participants with fractures were older, had higher blood pressure and higher levels of cholesterol, and smoked more. Except for diastolic blood pressure, differences were similar in men and women when analyzed separately.

During the course of 20 years, 143 of the participants sustained a total number of 258 fractures of osteoporotic origin. Hip fracture was the most common type of fracture among the participants and corresponded to 29% (of which 31% occurred in men) of the total number of fractures. Leg and wrist fractures corresponded to 15%, vertebrae fractures 14%, upper arm and ankle fractures 8%, and rib fractures made up 5% of the total number of fractures among the participants. None of these types of fractures differed between the sexes. Among male participants, 61% sustained a single fracture, 20% sustained two fractures, and 19% sustained three or more fractures. For female participants, the corresponding figures were 66%, 21%, and 13%, respectively.

The HR for a one step increase in SD of risk for fracture during 20 years was 1.05 (CI 1.03–1.07; $p<0.001$) for current age, 2.63 (CI 1.48–4.66; $p<0.001$) for previous fracture, 1.87 (CI 1.33–2.62; $p<0.001$) for smoking, 1.07 (CI 1.01–1.13; $p=0.014$) for coffee consumption in models including current age, sex, and time since baseline examination. When these factors were analyzed in a Poisson model together with current time, age, and gender, coffee consumption lost its significance. The HR for total cholesterol was 1.17 (CI 1.02–1.34; $p=0.029$) and BMI had an HR of 0.95 (CI 0.90–1.00; $p=0.046$). The β coefficients

Table 1 Anthropometric data, life style factors, blood pressure, lipids, and fibrinogen in men and women with and without osteoporotic fracture (mean ± SD)

	With fracture (N=143)	Without fracture (N=1,253)	p value
Fracture, N	258 (89 men, 96 women)	0	
Fracture/subject, N	1.8	0	
Age, years	48.7±11.3	44.1±11.3	<0.05
Height, cm	171.0±8.8	172.0±9.0	Ns
Weight, kg	71.5±13.1	72.3±13.2	Ns
Body mass index, kg/m^2	24.4±3.65	24.4±3.64	Ns
Waist, cm	86.5±11.8	85.1±11.9	Ns
Hip, cm	98.0±8.0	98.7±32.0	Ns
Waist/hip ratio	0.88±0.09	0.86±0.09	Ns
Smoke, 1–3, current, ex-smokers, non-smokers	1.59±0.57	1.73±0.53	<0.01
Number of cigarette equivalents/day, N	14.4±7.6	15.1±8.2	Ns
Physical activity at work, 1–4, low to high	2.0±0.9	2.1±0.9	Ns
Leisure time physical activity, 1–4, low to high	2.3±0.6	2.0±0.6	Ns
Stress, grade 1–6, low to high	3.1±1.4	3.2±1.4	Ns
Coffee, cups/day	4.5±2.4	4.2±2.8	Ns
Total cholesterol, mmol/l	6.36±1.33	5.88±1.24	<0.001
Triglycerides, mmol/l	1.31±0.83	1.24±0.92	Ns
Fibrinogen, g/l	2.81±0.73	2.73±0.72	Ns
Systolic blood pressure, mmHg	131.2±18.9	127.0±19.1	<0.01
Diastolic blood pressure, mmHg	82.8±10.8	80.5±10.6	<0.01
Hypertension, N (%)	11 (7.6)	61 (4.9)	Ns
Diabetes, N (%)	5 (3.5)	25 (1.9)	Ns

A brief comparison between the groups (p value) is listed unadjusted for age and follow-up time

for the factors significant for osteoporotic fracture in a multivariable context are listed in Table 2. As can be seen, gender was not significant. Less than a quarter of the cohort was postmenopausal. HRT did not influence the analysis.

LDL cholesterol and HDL cholesterol were included in multivariate analyses yielding HRs of 1.16 (CI=0.99–1.35, p=0.0589) and 1.52 (CI=0.99–2.34, p=0.0551), respectively. Total cholesterol was also a risk factor for myocardial infarction (n=56 in men and n=20 in women) in men (N=676; p=0.033) and in women (N=720, p=0.0012).

Table 2 Hazard function ($e^{(\beta 0+\beta 1\times\text{current age}+\beta 2\times\text{current time}+\beta 3\times\text{sex}+\beta 4\times\text{BMI}+\beta 5\times\text{cholesterol})}$) of osteoporotic fracture estimated by Poisson regression

Variable	β	SE	p value
Constant	−7.874	0.751	
Current age	0.047	0.009	<0.001
Current time (years)	0.020	0.019	0.30
Sex (0=men, 1=women)	0.287	0.177	0.10
BMI	−0.0527	0.026	0.046
Cholesterol	0.155	0.071	0.029

The standard deviations of BMI (3.64) and cholesterol (1.26) yield gradients of risk per SD of 1.21 ($e^{(0.0527\times 3.64)}$) and 1.22 ($e^{(0.155\times 1.26)}$), respectively

SE standard error

How the predictive power of a variable changes with time cannot be included in Cox's proportional hazard model since the interaction contradicts the proportional hazard condition [9]. Instead, analysis of how the predictive power

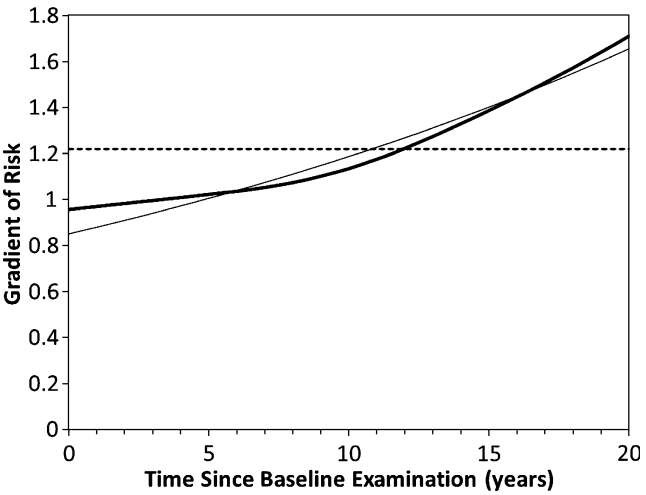

Fig. 1 Serum total cholesterol as an osteoporotic fracture predictor as a function of time. Cox's proportional hazard model shows the risk gradient as being constant over time (*dotted line*). Two Poisson regression models are shown for the gradient of risk per one standard deviation of serum total cholesterol; in the first model, the logarithm is linear as a function of time (*thin solid line*), and the second model is based on smoothing spline functions (*thick solid line*)

of a variable changes with time requires a hazard function that includes the current variable and its interaction with time since the measurement. Figure 1 shows the gradient of risk per one standard deviation of serum total cholesterol as a function of time. The Poisson regression model, in which the logarithm of the hazard ratio is linear as a function of time (Fig. 1, thin solid line), yields a significant increase of the gradient of risk by time (p=0.0377). A more precise assessment of the interaction using smoothing spline Poisson regression [10] confirms that the predictive power of serum total cholesterol improves with time (Fig. 1, thick solid line). A simpler Poisson model or Cox's model, in which the gradient is constant over time, is shown for comparison (Fig. 1, dotted line, and Table 2).

Discussion

This study confirms well-known risk factors for osteoporotic fracture, and underscores the importance of an unbiased randomly selected population. It also uniquely identifies serum total cholesterol as an independent osteoporotic fracture risk factor whose predictive power improves with time. Consequently, unless the number of participants is extremely large, only studies with long follow-up times will be able to assess the true power of cholesterol as an osteoporotic fracture risk factor. This may explain why long-term effects of cholesterol on osteoporotic fractures has been largely neglected to date. The existing body of literature is also very difficult to evaluate since studies aiming to investigate cholesterol as an independent osteoporotic fracture risk factor [11, 12], as well as lipid-lowering studies [13, 14], hitherto have been based on follow-up times of maximally 5 years. Moreover, to minimize effects of intra-individual fluctuations in cholesterol, a weighted sum of cholesterol values, recorded over a longer period of time, is preferred to a single time point measurement (like the baseline examination value used in this study). Future ad hoc studies aiming to clarify role(s) of cholesterol as a risk factor for osteoporotic fractures thus require longer follow-up times as well as the recording of cholesterol levels at regular intervals over a longer period of time.

The current study corroborates our recent finding that hard cardiovascular endpoints are strong predictive risk factors for hip fractures among elderly men [3]. Thus, serum total cholesterol is a risk factor not only for vascular-associated diseases, but also for osteoporotic fractures, perhaps pointing towards a common long-term origin of cardiovascular disease and osteoporosis [15]. In addition to incidence of myocardial infarction, aorta calcification may be another potential marker of arteriosclerosis.

An indirect association between serum total cholesterol and bone mineral density, which has been considered a strong determinant of osteoporotic fractures, has been demonstrated [15–19]. Some authors concluded this association to be a result of estrogen deficiency rather than cholesterol per se, albeit prospective studies remain to be done. The present longitudinal study supports the notion that serum total cholesterol has a role in causing osteoporotic fractures independently of gender and HRT. A previous report found that serum total cholesterol increases with age independently of gender [4], and moreover that general lipid-lowering statins decrease the risk for hip fracture independently of gender [20].

This is a first attempt to prospectively support the above mentioned cross-sectional and case-control studies. A limitation of the current study is that mechanisms that explain how serum total cholesterol increases the risk factor for osteoporotic fractures remain unknown. The study also lacks sufficient power to prove causal relations, i.e., whether cholesterol causes myocardial infarctions, which in turn may increase the risk of falling, or other more specific mechanisms as local damage to the bone. Strengths of this study are that the cohort was young, treatment naïve, and subject to a long follow-up time with hard endpoints.

In conclusion, our study identifies serum total cholesterol as an independent long-term osteoporotic fracture risk factor whose predictive power improves with time. This finding suggests that cholesterol is more central in causing osteoporotic fractures than previously thought, and warrants further studies.

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Conflicts of interest None.

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